Particulate Air Pollutants and Airway Inflammation

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Particulate air pollutants emanating from traffic and various industries are related to allergic airway disorders including asthma. Particulate air pollutants inhalation directly induces lung inflammation to allergens or respiratory viral infection as an adjuvant by macrophages and epithelial cells. Inhalation of particulate air pollutants aggravates respiratory symptoms in patients with chronic airway diseases, but the mechanisms underlying this response remain poorly understood. Diesel exhaust particles induced airway hyperresponsiveness and Ym mRNA expression via a Th2 cell-biased response, which synthesized by activated macrophages are homologous to chitinase and have chitinase activity. Alveolar macrophages play an important role in particles-induced airway and lung inflammation via direct production of IL-13. Treatment of epithelial cells with bovine serum albumin coated titanium dioxide particles altered 20 protein spots on the two-dimensional gel, and these were then analyzed by nano-LC-MS/MS. These proteins included defense-related, cell-activating, and cytoskeletal proteins implicated in the response to oxidative stress. TiO2 treatment was found to increase the amount of mRNA for macrophage migration-inhibitory factor (MIF). MIF was expressed primarily in epithelium and was elevated in lung tissues and bronchoalveolar lavage fluids of TiO2-treated rats as compared with sham treated rats. Carbon black and diesel exhaust particles also induced expression of MIF protein in the epithelial cells. We attempt to offer insight into how particles may influence airway inflammation.

Keywords: *Air pollution; Lung; Inflammation*

Introduction

Growing epidemiologic evidences have indicated that inhalation of airborne particulate matters (PM) is associated with adverse health outcome of increasing respiratory and cardiac mortality and morbidity.[1,2] Chronic obstructive pulmonary disease patients living in communities exposed to high levels of air pollution have faster rates of decline in lung function than those living in areas with low pollution.[3] The level of environmental particles is also positively correlated with exacerbation of asthma.[4] During the last decade, the composition of air pollution has changed from a classical type 1 pollution, consisting of SO_2 and large dust particles to modern type II pollution, characterized by oxides of nitrogen, organic compounds, ozone and ultra fine particles in developed countries.[5] Airborne particulate matter less than 10 μm in aerodynamic diameter (PM 10) is a complex mixture of materials having a carbonaceous core and associated materials such as organic compounds, acids, and fine particles of metals.[6]

PM10 or diesel extract particles (DEP) augment lung

inflammation by inhalant allergens or respiratory viral infection via acting as an adjuvant. The response may lead to enhancement of already existing allergies or IgE response to neoallergens and susceptibility to respiratory infection. This adjuvant effect is exerted by the enhanced production of inflammatory Th2 and/or Th1 cytokines.[7,8] In animal experiments and human studies, several cytokines and CC chemokines including IL-4, IL-5, IL-13, GM-CSF, RANTES, MCP-3, MIP-1 are increased when lymphocytes and macrophages/monocytes are co-stimulated with particulates in the presence of specific allergens.[9-11] The immune responses can be modified in different ways according to the types of particulates. DEP favor Th2 while asbestos fiber and carbon black particle pulsed-macrophages up-regulate both of Th1 cytokines and Th2 cytokines produced by autologous lymphocyte stimulated by antigen.[10]

In addition to the adjuvant effects, inhaled inert particles cause a spectrum of pulmonary responses, ranging from minimal changes to marked acute and chronic inflammation.[11] The inflammation inducing effects of PM 10 had been demonstrated on experimental animal studies following direct instillation into the lung, until the human study showed the pulmonary effects after experimental exposure of particulate matter.[12,13] Clinically, PM 10 particles are thought to provoke airway inflammation via the release of mediators that are capable of exacerbating lung disease in susceptible individuals[14] and even a single exposure compromises a host's ability to handle ongoing pulmonary infections.[15] The fine and ultrafine particles directly stimulate macrophages and epithelial cells to produce inflammatory cytokines such as TNF-α, TGF-β1, GM-CSF, PDGF, IL-6, and IL-8[16-19] and reactive oxygen species.[20] All of these cytokines are responsible for acute and chronic inflammation in the lung. However, there is a lack of evidence to reveal that particulates can directly induce Th2 like cytokines including IL-4 and IL-13.

TiO_2 particles are a PM10 component found in dusty workplaces in industries involved in the crushing and grinding of the mineral ore rutile.[21] Fifty percent of TiO_2-exposed workers have respiratory symptoms accompanied by reduction in pulmonary function.[22] Because acute and chronic exposures to TiO_2 particles also induce inflammatory responses in the airways and alveolar spaces of rats,[23-26] TiO_2-treated rats are a good model for the study of the human epithelial response to PM10 particles.

Proteomics offers a unique means of analyzing the expressed genome, and it has been successfully used to examine the generation of oxidative stress at the cellular level.[27] In addition to revealing protein modifications, this approach can also be used to look at changes in protein expression levels.[28]

In our study we developed a mouse model exposed to DEP, and the effect of DEP exposure, as an air pollutant, on Ym1 and Ym2 mRNA expression was examined.[29] And we demonstrate that alveolar macrophages produce IL-13 in response to fine TiO_2 particles at protein and mRNA levels in vivo and in vitro[24] and also, we adopted a proteomics approach to identify the protein changes that occur in epithelial cells in response to exposure to bovine serum albumin-coated TiO_2 particles.[30] Two dimensional electrophoresis data were validated by RT-PCR, and then the data were also proved using an animal model.

In this article, we attempt to offer insight into how particles may influence airway inflammation in our data of epidemiologic and in vitro, and in vivo studies.

Epidemiologic Studies

It is well known that increased air pollution emanating from traffic and various industries has resulted in an increase in the incidence of allergic diseases. Acute exposure to air pollution is associated with increased respiratory symptoms and decreases in lung function in children. Chronic exposure to increased levels of respirable particles, SO_2, and NO_2 is associated with up to threefold increase in nonspecific respiratory symptoms,

such as chronic cough, asthma, and chronic airway diseases. Exposure to heavy traffic leads to significant increases in respiratory symptoms, while no clear effect on the inception of asthma has been documented. Outdoor air pollution levels have been associated with adverse effects in subjects with asthma,[31] and exposure to traffic-related air pollution, in particular diesel-exhaust particles, may lead to reduced lung function in children living near major motorways.[32]

The prevalence of airway hyperresponsiveness (AHR) has increased over the last few decades and it is generally believed that this is because of environmental factors. Air pollution is convincingly associated with many signs of asthma aggravation, including pulmonary function decrements, increased AHR, visits to emergency departments, hospital admissions, increased medication use, and reported symptoms; it is also associated with inflammatory changes, interactions between air pollution and allergen challenges, and immune symptom changes.[33] A significant association between traffic-related air pollution and wheeze has also been reported in children,[34,35] and exposure to diesel exhaust particles may reduce lung function in children living near motorways. Long-term exposure to ambient ozone has been associated with the development of asthma in adult males.[36] Peter et al.[37] observed significant, physiologically important losses in FVC, FEV1, peak expiratory flow rate, and maximal midexpiratory flow associated with pollution levels in females in 3293 Southern California public schoolchildren and adolescents. Kreit et al.[38] found an enhanced response to a methacholine provocative challenge only in the subjects with asthma after a 2-h exposure to 0.4 ppm ozone. In a meta-analysis, Folinsbee[39] found a small, but significant, trend to increased AHR after controlling for NO_2 exposure in subjects with asthma. A significant relationship was found between the mean annual SO_2 level and the prevalence of asthma in adults aged $25 \sim 29$ years.[40] In asthmatic children attending school in urban Amsterdam, black smoke was the most important air pollution indicator associated with acute changes in lung function, respiratory symptoms, and medication use.[41] We have surveyed 670 schoolchildren in one of polluted area. 42 All of the children had normal pulmonary function, while 257 (38.3%) had AHR. A significantly greater proportion of children had AHR in the polluted area [45.0% (138/306), 6.50±0.48] than in the rural [31.9% (52/163), 9.84±0.83] or coastal [33.3% (67/201), 7.17±0.68] areas. Schoolchildren with atopy had a lower PC20 than those without atopy (5.98± 0.60 vs. 8.15±0.45, p<0.001). In the multiple logistic regression model, positive allergy skin test and living in the polluted area near the chemical factory were independently associated with AHR (odds ratio for location=2.4875, CI 1.6542~3.7406, P<0.01; odds ratio for allergy skin test=1.5782, CI 1.1130~2.2379, p< 0.05), when adjusted for sex, parents' smoking habits, age, body mass index, nose symptoms, and lung symptoms. Our results suggest that air pollution in the area near the polluted area affects the development of AHR, and that controlling air pollution is important for preventing the development of asthma.

Effect of Particles Exposure on AHR and Airway Inflammation

We hypothesized that DEP play a role in increasing asthma prevalence, although a causal relationship has yet to be established. To investigate the effects of DEP exposure on airway inflammation and AHR, we exposed and challenged DEP in mice. Airway responsiveness was measured in unrestrained, conscious mice 1 day after the last challenge, as previously described.[43]

Mice were placed in a barometric plethysmographic chamber (All Medicus Co., Seoul, Korea), and baseline readings were taken and averaged for 3 min. Increasing concentrations of aerosolized methacholine, from 2.5 to 50 mg/ml, were nebulized through an inlet of the main chamber for 3 min. Readings were taken and averaged for 3 min after each nebulization and enhanced pause (Penh) was determined. Penh, calculated as (expiratory

time/relaxation time−1) × (peak expiratory flow/peak inspiratory flow), according to the manufacturer's protocol, is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and a function of the timing of expiration. Penh is used as a measure of airway responsiveness to methacholine. Results are expressed as the percentage increase of Penh following challenge with each concentration of methacholine, where the baseline Penh (after saline challenge) is expressed as 100%. Penh values averaged for 3 min after each nebulization were evaluated.

To investigate the role of DEP in induction of AHR[29], in a modification of the classical OVA sensitization and challenge model, mice were exposed to intranasal DEP and challenged with aerosolized DEP on days 6~8. Penh was measured on day 9 and sample collection occurred on day 10. The negative controls included animals receiving intranasal saline followed by aerosolized saline on days 6~8. Delivery of aerosolized DEP following exposure with intranasal DEP induced a significant increase in methacholine-induced Penh, indicating AHR.

Molecular Mechanisms in vitro and in vivo Studies

Chitin is a common element in organisms including parasites, fungi, and bacteria, but does not occur in mammalian tissues,[44] allowing for selective antimicrobial activity of chitinase. Ym1 and Ym2 synthesized by activated macrophages are homologous to chitinase and have chitinase activity.[45,46]

Ym1 is involved in allergic peritonitis through the IL-4/STAT 6 signal transduction pathway.[47] Zhu et al.[48] reported that acid mammalian chitinase may be an important mediator of IL13-induced responses in Th2-dominated disorders such as asthma. An association between acid mammalian chitinase polymorphisms and asthma further supports the involvement of acid mammalian chitinase in asthma development.[49] The regulation and function of chitinase has not been well explored in air pollution asthma models. Therefore we developed a mouse model exposed to DEP, and the effect of DEP exposure, as an air pollutant, on Ym1 and Ym2 mRNA expression was examined. Ym1 was one of the most highly induced IL-4 target genes, exhibiting a 70-fold or greater increase in induction in multiple macrophage populations. Ym1 was most expressed in the spleen and lungs, with lower expression in the thymus, intestine, and kidney, whereas Ym2 was most expressed in the stomach, with lower levels of expression in the thymus and kidney.[50,51] The conservation of STAT6 sites probably accounts for the similar, striking induction of Ym1 and Ym2 expression in Th2-type environments. We examined the possibility that Ym1 and Ym2 may be expressed in a murine model when exposed to DEP and found that TH2-biased BALB/c mice exposed intranasally with DEP, followed by a DEP challenge, up-regulated lung expression of Ym1 and Ym2 transcripts in comparison with mice neither exposed nor similarly challenged (Fig. 1).

Nitric oxide (NO) is a short-lived molecule that causes vasodilation and bronchodilation.[52,53] We demonstrated that concentrations of NO metabolites increased with asthma severity in the tracheo-bronchial secretions of asthmatic subjects, and that NO metabolites in sputum

Fig. 1. The expression of Ym1 and YM2 mRNA in the lungs of DEP-treated and control mice. Lung tissues from DEP-treated and control mice were stained with monoclonal antibodies directed against YM1 and YM2.

are a more valuable indicator for monitoring asthmatic airway inflammation than those in serum.[54,55] The Nitric oxide synthase (NOS) isoforms play different roles in airway inflammation after ozone exposure.[56] The nitrite concentration[29] in BAL fluids, which indicates the in vivo generation of NO in the airways, was significantly greater in the DEP exposure group than in the control group (52.3 ± 15.1 and 29.5 ± 5.7 μmol/l, $p<0.05$). The alveolar macrophages produced nitrite during in vitro stimulation with DEP particles (50 μg/ml), with maximal induction at 4 h after stimulation (Fig. 2).
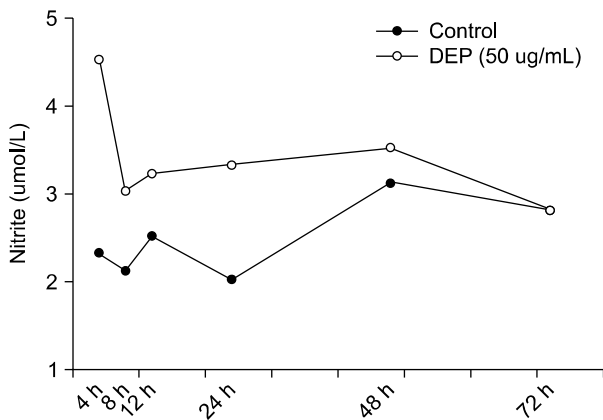

Fig. 2. Nitrite production by alveolar macrophages (Raw 264.7 cells) exposed to 50 μg DEP/ml. $p<0.05$ versus the control group.

We hypothesize that particles induce a Th2-like environment, with the overproduction of IL-4 and IL-13.[24] The expression of IL-13 mRNA transcripts was investigated using RT-PCR of lung tissue extracts that were obtained from TiO_2-treated and sham-treated rats. The levels of IL-13 mRNA expression were significantly increased in the lungs 24 hours after treatment with TiO_2 particles, compared to those of sham-treated rats. The levels of IL-13, as measured by ELISA, were significantly increased in the BAL fluids of TiO_2-treated rats 72 hours after treatment (n=8), as compared to those of sham-treated rats (n=8) (P= 0.03). In order to investigate the time- and dose- dependency of macrophage IL-13 production, purified alveolar macrophages were stimulated with 1, 10, and 40 μg/ml TiO_2 for 24, 48, and 72 hours (n=6 in each experiment). The control group (n=6) consisted of untreated alveolar macrophages. The IL-13 levels in the supernatants of the macrophage cultures were measured by ELISA. Macrophages that were cultured for 48 hours with TiO_2 produced IL-13 in a dose-dependent manner (Fig. 3A). TiO_2 concentrations >10 μg/ml significantly enhanced IL-13 production when compared with the control group. The production of IL-13 protein was
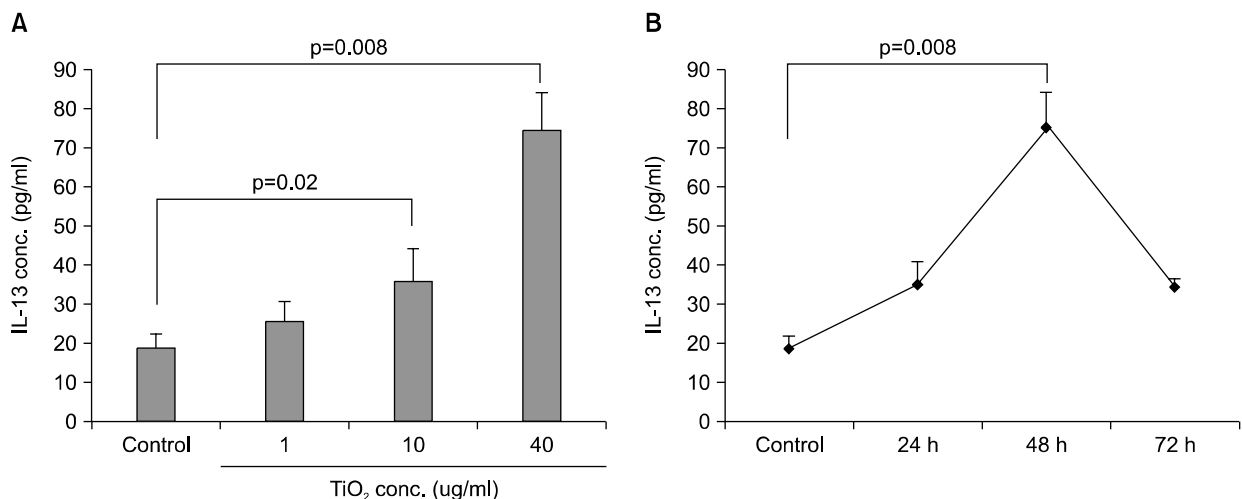

Fig. 3. Time and dose responses of IL-13 production by macrophages exposed to TiO_2 particles. Purified alveolar macrophages stimulated with 1, 10, and 40 μg/ml TiO_2 for 24, 48, and 72 hours (n=6 in each experiment). The control group (n=6) consisted of unstimulated alveolar macrophages. The IL-13 in the 48-hour culture supernatants is produced in a dose-dependent manner following TiO_2 treatment (A). TiO_2 concentrations higher than 10 μg/ml significantly enhance IL-13 production when compared with the control group. The production of IL-13 protein is increased in a time-dependent manner and peaks 48 hours after TiO_2 stimulation (B). The results are expressed as means$\pm$SEM.

Group 1
1. ATP6V1B2
2. K6A
3. MIF

Group 2
4. HSPD1
5. UVBL2
6. PCNA
7. TALDO1
8. CLIC1

Group 3
9. MCM7
10. CAPN1
11. RNH1
12. VIM
13. PSMD9
14. ACTR2
15. PSMD14

Group 4
16. PRP19
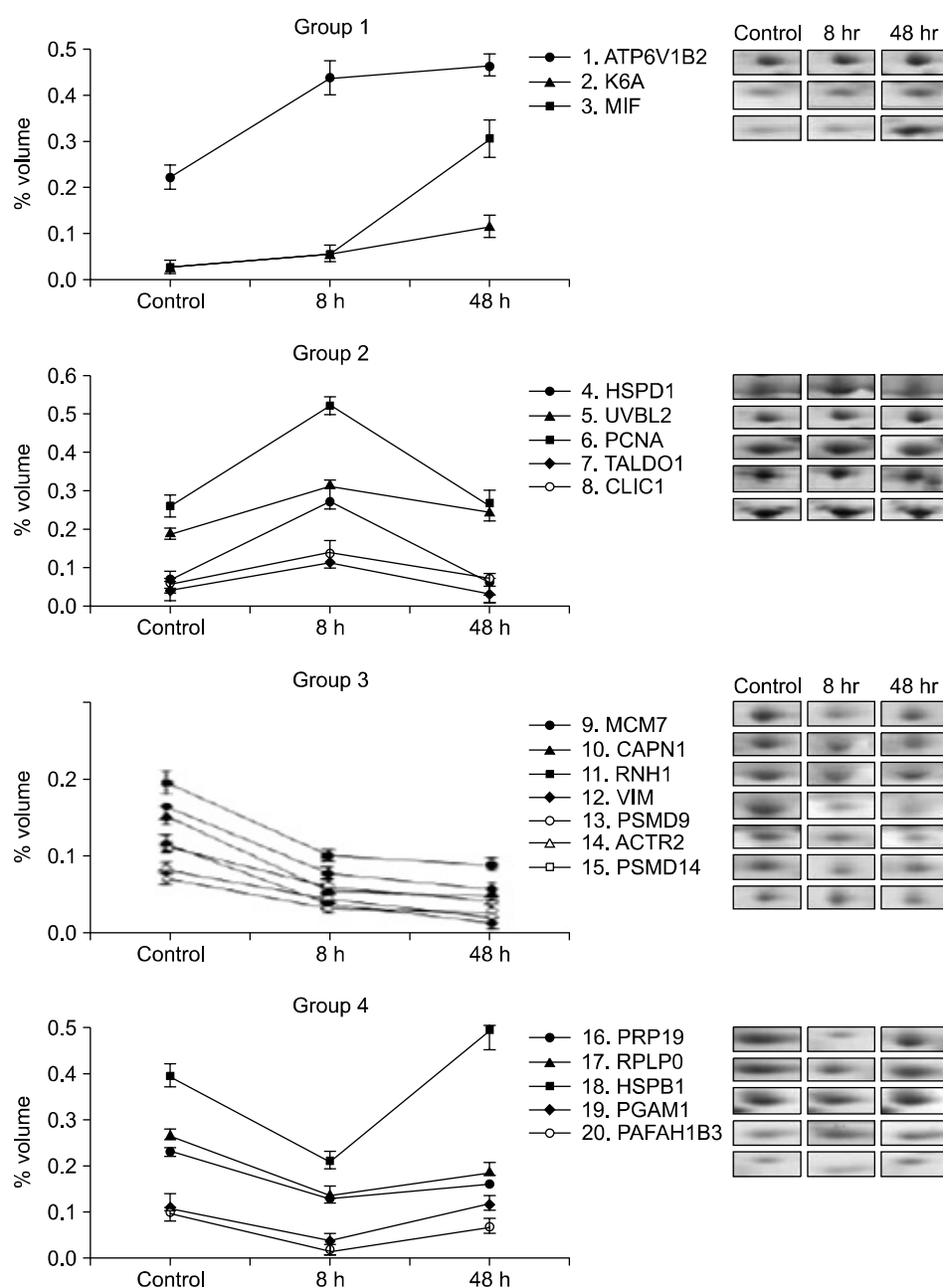17. RPLP0
18. HSPB1
19. PGAM1
20. PAFAH1B3

Fig. 4. Cluster analysis of 20 proteins with significant differential expression (>2-fold change) at 8 or 48 h caused by TiO2 treatment of BEAS-2B epithelial cells. The expression profiles of the individual proteins were classified by cluster analysis. Protein names (National Center for Biotechnology Information (NCBI)) are displayed for each cluster.

increased in a time-dependent manner and peaked 48 hours after TiO2 stimulation (Fig. 3B). Using immuno-histochemical staining, IL-13-positive cells were clearly identified as macrophages that had engulfed TiO2 particles. These data indicate that TiO_2-engulfing macrophages are the main source of IL-13 in TiO2 particle-induced inflammation of the lung. Taken together, our results suggest that alveolar macrophages may act as major effectors of innate immunity to modulate immune and inflammatory responses towards a Th2-like con-dition via the production of IL-13, as seen in the adaptive immune response.

Proteomics offers a unique means of analyzing the expressed genome, and it has been successfully used to examine the generation of oxidative stress at the cellular level. In addition to revealing protein modifica-tions, this approach can also be used to look at changes in protein expression levels. We[30] identified 20 proteins (Fig. 4) whose expression levels in the BEAS-2B cell line changed in response to TiO_2 particle exposure
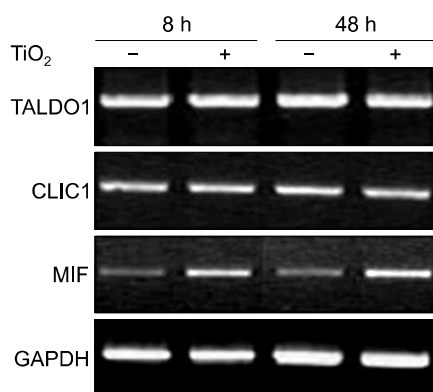
Fig. 5. Semiquantitative RT-PCR analysis of TALDO1, CLIC1, and MIF mRNA expression. The levels of mRNA for TALDO1, CLIC1, and MIF were estimated in extracts of BEAS-2B cells stimulated with TiO2 particles (20 μg/10^5 cells). Expression levels were determined by densitometry and normalized to the level of GAPDH mRNA.

using proteomics. These proteins include defense-related, cell-activating, and cytoskeletal proteins implicated in the response to oxidative stress, and they can be classified into four groups according to the pattern of their TiO2-induced change in expression over time. One of these proteins, migration-inhibitory factor (MIF, Fig. 5), was induced at the transcriptional level by stimulation of cells with any one of three different particulate molecules, and expression of MIF protein was increased in the lungs of TiO2-instilled rats. These results indicate that some of these proteins may serve as mediators of, or markers for, airway disease caused by exposure to PM.

Conclusion

Epidemiological survey and animal experimental studies all together suggest that particulates air pollutants are involved in the pathogenesis of airway inflammation and aggravate respiratory symptoms. The controlling air pollution is important for preventing the development of airway diseases. In vitro and in vivo studies should be needed to further delineate the role of particulate air pollutants in airway diseases and related molecular mechanisms.

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